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Women Produce Fewer but Triglyceride-Richer Very Low-Density Lipoproteins than Men

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Context: Very low-density lipoproteins (VLDL) are a major risk factor for cardiovascular disease. The concentrations of VLDL particles and VLDL-triglyceride (TG) in plasma are lower in women than men, but the mechanisms responsible for these differences are unclear.

Objective: The objective of the study was to investigate the effects of sex on VLDL-TG and VLDL-apolipoprotein B-100 (apoB-100) metabolism.

Experimental Design and Main Outcome Measures: We measured basal VLDL-TG and VLDL-apoB-100 kinetics by using stable isotope labeled tracers.

Setting/Participants: Twenty-six healthy, lean subjects (13 men, aged 29 ± 5 yr; 13 women, aged 28 ± 6 yr) were studied in the General Clinical Research Center at Washington University School of Medicine.

Results: VLDL-TG and VLDL-apoB-100 concentrations were less in women than men ($P < 0.05$). The secretion rate of VLDL-TG was approximately 70% greater ($P < 0.05$), whereas the secretion rate of VLDL-apoB-100 (*i.e.* VLDL particles) was approximately 20% less ($P < 0.05$) in women than men. The molar ratio of VLDL-TG and VLDL-apoB-100 secretion rates was therefore more than double ($P < 0.05$) in women than men. VLDL-TG plasma clearance rate was approximately 70% greater in women than men ($P < 0.05$), whereas VLDL-apoB-100 plasma clearance rate was not different between sexes. However, VLDL-TG and VLDL-apoB-100 mean residence times in plasma were both shorter (by 45 and 25%, respectively; $P < 0.05$) in women than men.

Conclusions: Increased VLDL-TG plasma clearance is responsible for lower VLDL-TG concentration, whereas decreased VLDL-apoB-100 secretion rate, combined with shorter VLDL-apoB-100 residence time in plasma, is responsible for lower VLDL-apoB-100 concentration in women than men. The greater molar ratio of VLDL-TG and VLDL-apoB-100 secretion rates suggests that the liver in women secretes fewer but TG-richer VLDL particles than the liver in men. (***J Clin Endocrinol Metab* 92: 1311–1318, 2007**)

WOMEN OF REPRODUCTIVE age have a lower risk of cardiovascular disease (CVD) than age-matched men (1). Sex differences in very low-density lipoprotein (VLDL)-triglyceride (TG) and VLDL-apolipoprotein B-100 (apoB-100) metabolism likely contribute to the lower CVD risk in women because VLDL particles and plasma TG concentrations are risk factors for CVD (2–4), and women have lower concentrations of VLDL and TG in plasma than men (5–8). Moreover, sex differences in total fasting plasma TG concentrations are entirely attributed to lower VLDL-TG concentration (9, 10).

VLDL are produced and secreted by the liver and are composed of TGs (~50–70%), cholesterol and cholesteryl esters (~20–40%), phospholipids (~5–10%), and the apolipoproteins B-100, C, and E. The synthesis, assembly, and secretion of VLDL-TG and VLDL-apoB-100 within hepatocytes are both independent and interactive processes. VLDL-apoB-100 provides the structural framework of the VLDL particle and permits the incorporation of TGs, cholesterol and cholesteryl esters, and other apolipoproteins before VLDL secretion occurs (4, 11). Only one apoB-100 molecule is present in each VLDL particle (12), so VLDL-apoB-100 kinetic rates reflect the metabolic behavior of the VLDL particle. In contrast, the number of TG molecules present in VLDL can vary considerably (3, 11). The relationship between hepatic VLDL-TG and VLDL-apoB-100 secretion provides an index of the average TG content and therefore of the average size of VLDL particles secreted by the liver. Although changes in VLDL-TG and VLDL-apoB-100 secretion rates are similar in response to certain stimuli, *e.g.* free fatty acid (FFA) and insulin infusion (13, 14), there is also evidence that the secretion of VLDL-TG and VLDL-apoB-100 is independently regulated, *e.g.* in response to manipulation of dietary macronutrient composition, weight loss, and endurance exercise (15–18).

We recently found differences in VLDL-TG secretion and VLDL-TG plasma clearance rates between lean men and lean women (19). However, the effect of sex on the relationship between VLDL-TG and VLDL-apoB-100 fluxes during normal physiological conditions has not been investigated. Understanding the relationship between VLDL-TG and VLDL-apoB-100 metabolism has important physiological and clinical implications. TG removal from large, TG-rich VLDL particles via lipoprotein lipase (LPL) is more efficient than TG removal from small, TG-poor particles (20, 21). Further-

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Abbreviations: apoB-100, Apolipoprotein B-100; CVD, cardiovascular disease; FFA, free fatty acid; FFM, fat-free mass; FTR, fractional turnover rate; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment insulin resistance; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; LPL, lipoprotein lipase; MRT, mean residence time; Ra, rate of appearance; TG, triglyceride; TTR, tracer to tracee ratio; VLDL, very low-density lipoprotein.

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more, large VLDL and their lipolytic products have been associated with insulin resistance and atherosclerosis in humans (2–4). The purpose of the present study was to evaluate VLDL-TG and VLDL-apoB-100 kinetics, by using stable isotope-labeled tracers and mathematical modeling, in healthy, lean men and women.

Subjects and Methods

Subjects

Thirteen lean men and 13 lean women participated in the study (Table 1). Some but not all data presented here have previously been reported when basal FFA, VLDL-TG, and VLDL-apoB-100 kinetics were assessed for other purposes (16, 22). All subjects were considered to be in good health after completing a medical evaluation, which included a history and physical examination and standard blood tests. No subjects smoked cigarettes or were taking medications. We did not control for menstrual cycle phase in women because we have previously demonstrated that basal plasma FFA, VLDL-TG, and VLDL-apoB-100 kinetics are not different during the follicular and luteal phases of the menstrual cycle (22). Written informed consent was obtained from all subjects before their participation in the study, which was approved by the Human Studies Committee and the General Clinical Research Center Advisory Committee at Washington University School of Medicine (St. Louis, MO).

Experimental protocol

Total body fat mass and fat-free mass (FFM) were assessed by dual-energy x-ray absorptiometry (Delphi-W densitometer; Hologic, Waltham, MA). Total abdominal, intraabdominal, and sc abdominal fat areas were determined by magnetic resonance imaging on a 1.5T scanner (Siemens, Iselin, NJ). Eight 10-mm-thick slice images were obtained beginning at the L_4–L_5 interspace and analyzed for sc abdominal and intraabdominal fat cross-sectional areas with Analyze 6.0 software (Mayo Foundation, Biomedical Imaging Resource, Rochester, MN); the areas from serial images of the abdomen were averaged.

To determine whole-body FFA, VLDL-TG, and VLDL-apoB-100 kinetics, each subject completed a stable isotope-labeled tracer infusion study. Participants were instructed to adhere to their regular diet and to refrain from exercise for 3 d before the study. Subjects were admitted to the General Clinical Research Center the afternoon before the tracer infusion study. At 1930 h, they consumed a standard meal, containing 12 kcal/kg body weight (55% of total energy from carbohydrate, 30% from fat, and 15% from protein) and then fasted (except for water) and rested in bed until completion of the study the next day. At 0530 h the following morning, one catheter was inserted into a forearm vein to administer stable isotope-labeled tracers, and a second catheter was inserted into a vein in the contralateral hand, which was heated to 55 C with a thermostatically controlled box to obtain arterialized blood samples. Catheters were kept open by infusing 0.9% NaCl solution (30 ml/h). At 0700 h (time = 0), after blood samples for the determination of plasma substrate and hormone concentrations and background glycerol, palmitate and leucine tracer to tracee ratios (TTR) in plasma and VLDL-TG and apoB-100 were obtained, a bolus of [1,1,2,3,3-$^2H_5$]glycerol (75 μmol/kg), dissolved in 0.9% NaCl solution, was administered through the catheter in the forearm vein, and constant infusions of [2,2-$^2H_2$]palmitate (0.03 μmol/kg·min), dissolved in 25% human albumin solution, and [5,5,5-$^2H_3$]leucine (0.12 μmol/kg·min; priming dose: 7.2 μmol/kg), dissolved in 0.9% NaCl solution, were started and maintained for 12 h. Blood samples were collected at 5, 15, 30, 60, 90, and 120 min and then every hour for 10 h to determine glycerol and palmitate TTR in plasma and VLDL-TG, and leucine TTR in plasma and VLDL-apoB-100.

TABLE 1. Anthropometric characteristics of study participants

	Men	Women	*P* value
n	13	13	
Age (yr)	29 ± 5	28 ± 6	0.685
Height (cm)	179 ± 5	167 ± 7	<0.001
Body mass index (kg/m^2)	22.0 ± 1.7	22.0 ± 1.5	0.983
Body weight (kg)	71 ± 7	61 ± 8	0.003
FFM (percent body weight)	87 ± 5	73 ± 4	<0.001
Fat mass (percent body weight)	13 ± 5	27 ± 4	<0.001
Abdominal fat area (cm^2)	123 ± 59	161 ± 58	0.119
Intraabdominal fat (percent total abdominal fat area)	37 ± 17	25 ± 10	0.036
Subcutaneous fat (percent total abdominal fat area)	63 ± 17	75 ± 10	0.036

Values are means ± SD.

Sample collection and analyses

To determine glucose concentration, blood was collected in tubes containing heparin; plasma was separated by centrifugation and analyzed immediately. All other blood samples were collected in chilled tubes containing sodium EDTA. Samples were placed on ice, and plasma was separated by centrifugation within 30 min of collection. Aliquots of plasma were kept in the refrigerator for immediate isolation of VLDL and measurement of plasma apoB-100 concentration. The remaining plasma samples were stored at −80 C until final analyses were performed.

The VLDL fraction was prepared as previously described (17). Briefly, approximately 1.5 ml of plasma were transferred into OptiSeal polyallomer tubes (Beckman Instruments, Palo Alto, CA), overlaid with a NaCl/EDTA solution (1.006 g/ml) and centrifuged at 100,000 × *g* for 16 h at 10 C in an Optima LE-80K preparative ultracentrifuge equipped with a type 50.4 Ti rotor (Beckman Instruments). The top layer, containing VLDL, was removed by tube slicing (CentriTube slicer; Beckman Instruments). Aliquots of the VLDL fraction were set aside for measuring VLDL-apoB-100 concentration immediately after collection; the remaining samples were stored at −80 C until final analyses were performed.

Plasma testosterone, progesterone, and 17β-estradiol concentrations were measured by using ELISA kits (IBL Immuno-Biological Laboratories GmbH, Hamburg, Germany). Plasma glucose concentration was determined on an automated glucose analyzer (YSI 2300 STAT plus; Yellow Spring Instrument Co., Yellow Springs, OH). Plasma insulin concentration was measured with RIA (Linco Research, St. Louis, MO). Plasma FFA concentrations were quantified by gas chromatography (HP 5890 series II GC; Hewlett-Packard, Palo Alto, CA) after adding heptadecanoic acid to plasma as an internal standard (23). Total plasma TG and VLDL-TG concentrations were determined with a colorimetric enzymatic kit (Sigma Chemicals, St. Louis, MO). Total plasma apoB-100 and VLDL-apoB-100 concentrations were measured with a turbidimetric immunoassay (Wako Pure Chemical Industries, Osaka, Japan).

Plasma free glycerol, palmitate, and leucine TTRs, the TTRs of glycerol and palmitate in VLDL-TG, and the TTR of leucine in VLDL-apoB-100 were determined by gas chromatography-mass spectrometry (Agilent Technologies/HP 6890 series GC System-5973 mass selective detector; Hewlett-Packard) (17, 23). The heptafluorobutyryl derivative was formed for the analysis of glycerol in plasma and VLDL-TG, the tertiary-butyldimethylsilyl derivative was prepared for plasma leucine, and the *N*-heptafluorobutyryl *n*-propyl ester derivative was used for leucine in VLDL-apoB-100. Plasma free palmitate and palmitate in VLDL-TG were analyzed as methyl esters.

Calculations

Palmitate rate of appearance (Ra) in plasma was calculated by dividing the palmitate tracer infusion rate by the average plasma palmitate TTR value between 60 and 240 min during physiologic and isotopic steady-state; total FFA Ra was derived by dividing palmitate Ra by the proportional contribution of palmitate to total plasma FFA concentration (24). The homeostasis model assessment insulin resistance (HOMA-IR) index was calculated to assess whole-body insulin resistance (25).

A metabolic steady-state existed with regard to VLDL-TG and VLDL-apoB-100 kinetics because plasma VLDL-TG and VLDL-apoB-100 concentrations remained constant throughout the 12-h sampling period.

The fractional turnover rate (FTR) of VLDL-TG was determined by fitting the TTR time courses of free glycerol in plasma and glycerol in VLDL-TG to a compartmental model (26). The rate of VLDL-TG secretion (in micromoles per liter plasma per minute), which represents the amount of VLDL-TG secreted by the liver per unit of plasma, was calculated by multiplying the FTR of VLDL-TG (in pools per minute) by the concentration of VLDL-TG in plasma (in micromoles per liter). The plasma clearance rate of VLDL-TG (in milliliters plasma per minute), which is an index of the removal efficiency of VLDL-TG from plasma, was calculated by dividing the VLDL-TG secretion rate (in micromoles per minute) by the VLDL-TG concentration (in micromoles per milliliters). The mean residence time (MRT) of VLDL-TG (in minutes) was calculated as 1/FTR. The VLDL-TG MRT indicates the average time that TG secreted in the core of VLDL remain in the VLDL fraction in the bloodstream. A short VLDL-TG MRT indicates a quick removal of TG from circulating VLDL particles, whereas a long MRT suggests that VLDL-TG, after being secreted by the liver, remains in plasma without being removed for a considerable amount of time. VLDL-TG plasma clearance and VLDL-TG MRT refer to loss of TG from the VLDL fraction via all possible routes, including LPL-mediated hydrolysis, transfer to other lipoproteins [*e.g.* high-density lipoproteins (HDLs)] via neutral lipid exchange, or conversion to higher-density particles [*i.e.* intermediate-density lipoproteins (IDLs) and low-density lipoproteins (LDLs)].

The relative contribution of systemic plasma FFAs and nonsystemic fatty acids to total VLDL-TG production was calculated by the principle of isotopic dilution upon fitting the palmitate TTR in plasma and VLDL-TG to the compartmental model used for the calculation of VLDL-TG FTR (26). By fitting the isotopic enrichments of plasma free palmitate and palmitate in VLDL-TG to this model, one can derive a dilution factor that indicates the extent to which the plasma palmitate enrichment was diluted by unlabeled sources of palmitate before being incorporated into VLDL-TG. These unlabeled, nonsystemic fatty acids in VLDL-TG are derived from pools of fatty acids that are not labeled with tracer during the infusion period; this includes: 1) fatty acids released from preexisting, slowly turning over lipid stores in the liver and tissues draining directly into the portal vein, 2) fatty acids resulting from lipolysis of plasma lipoproteins that are taken up by the liver, and 3) fatty acids derived from hepatic *de novo* lipogenesis (13). The remaining fatty acids in VLDL-TG (systemic plasma FFAs) represent FFAs from the systemic circulation that are taken up by the liver and directly incorporated into VLDL-TG or temporarily incorporated into rapidly turning over intrahepatic and ip TG stores before incorporation into VLDL-TG.

The FTR of VLDL-apoB-100 was calculated by fitting the TTR time courses of free leucine in plasma and leucine in VLDL-apoB-100 to a compartmental model (17). The rate of VLDL-apoB-100 secretion, the plasma clearance rate of VLDL-apoB-100, and the MRT of VLDL-apoB-100 (indices of the secretion rate, plasma clearance rate, and MRT of VLDL particles) were calculated based on plasma VLDL-apoB-100 concentration and VLDL-apoB-100 FTR as described above for VLDL-TG. VLDL-apoB-100 clearance, an index of the removal efficiency of VLDL particles, and VLDL-apoB-100 MRT, an index of the time VLDL particles spend in the circulation, refer to the loss of the VLDL particle from the VLDL density range either through conversion to higher-density particles (*i.e.* IDL and LDL) or irreversible loss from plasma (*e.g.* direct uptake of the particle by tissues). A molecular mass of 512,723 g/mol for apoB-100 was used for unit conversions (27).

Statistical analysis

All data sets were tested for normality according to Kolmogorov-Smirnov. Data are presented as means ± SD for normally distributed variables; differences between men and women were examined by using multivariate ANOVA with Bonferroni's adjustment for multiple comparisons. Sex hormone concentrations were not normally distributed and are therefore presented as medians and quartiles; differences between men and women were examined by using the nonparametric Mann-Whitney *U* test. Associations between variables of interest (*i.e.* plasma FFA concentration and VLDL-TG and VLDL-apoB-100 secretion rates; molar ratio of VLDL-TG and VLDL-apoB-100 secretion rates and VLDL-TG MRT) were assessed with regression analyses. $P \leq 0.05$ was considered statistically significant.

Results

Plasma substrate and hormone concentrations

Plasma insulin and glucose concentrations and whole-body insulin sensitivity (HOMA-IR index) were not different in men and women (Table 2). Plasma FFA concentration was approximately 45% greater in women than in men, but the difference did not reach statistical significance (Table 2). VLDL-TG and VLDL-apoB-100 concentrations were much less in women than in men (Table 2).

FFA Ra

Total FFA Ra was not different in men and women (216 ± 61 and 244 ± 72 μmol/min, respectively, $P = 0.285$). However, FFA Ra adjusted for differences in body size between women and men (4.1 ± 1.4 and 3.0 ± 0.8 μmol/kg body weight per minute, respectively; $P = 0.032$) and FFA Ra in relation to FFM (5.5 ± 1.7 and 3.5 ± 1.0 μmol/kg FFM per minute, respectively; $P = 0.002$) and FFA volume of distribution (101 ± 31 and 64 ± 19 μmol/liter plasma per minute, respectively; $P = 0.002$) was 35–55% greater in women than men.

VLDL-TG and VLDL-apoB-100 kinetics

Average TTR time courses for glycerol and palmitate in VLDL-TG and leucine in VLDL-apoB-100 in men and women

TABLE 2. Fasting plasma substrate and hormone concentrations

	Men	Women	*P* value
Testosterone (nmol/liter)[a]	19.4 (17.3, 21.0)	1.4 (1.0, 2.1)	<0.001
17β-estradiol (pmol/liter)[b]	60 (49, 82)	155 (69, 263)	0.023
Progesterone (nmol/liter)[c]	0.8 (0.7, 1.1)	5.6 (0.9, 16.5)	0.027
Insulin (pmol/liter)[d]	30 ± 13	32 ± 19	0.813
Glucose (mmol/liter)	5.0 ± 0.3	4.9 ± 0.3	0.346
HOMA-IR index	1.13 ± 0.56	1.15 ± 0.72	0.940
FFAs (μmol/liter)	397 ± 154	574 ± 271	0.124
Total TG (mmol/liter)	0.73 ± 0.20	0.64 ± 0.15	0.180
VLDL-TG (mmol/liter)	0.38 ± 0.13	0.29 ± 0.09	0.036
Total apoB-100 (μmol/liter)	1.25 ± 0.21	0.93 ± 0.25	0.002
VLDL-apoB-100 (nmol/liter)	55 ± 16	32 ± 11	<0.001

Values are means ± SD or medians (quartiles).
[a] Conversion factor from nanograms per milliliter to nanomoles per liter: 3.467.
[b] Conversion factor from from picograms per milliliter to picomoles per liter: 3.671.
[c] Conversion factor from nanograms per milliliter to nanomoles per liter: 3.18.
[d] Conversion factor from microunits per milliliter to picomoles per liter: 6.0.

and a representative model fit to the data from one subject are shown in Fig. 1.

The FTR of VLDL-TG was 1.03 ± 0.58 pools per hour in women and 0.44 ± 0.11 pools per hour in men ($P = 0.001$); VLDL-apoB-100 FTR was 0.49 ± 0.16 pools per hour in women and 0.35 ± 0.07 pools per hour in men ($P = 0.011$).

The secretion rate of VLDL-TG was approximately 70% greater in women than men ($P = 0.023$; Fig. 2), whereas the secretion rate of VLDL-apoB-100 was approximately 20% less in women than in men ($P = 0.030$; Fig. 2). Therefore, the molar ratio of VLDL-TG and VLDL-apoB-100 secretion rates, an index of the TG content of newly secreted VLDL particles, was more than double in women than men ($P = 0.002$; Fig. 2).

The relative contribution of systemic plasma FFA and nonsystemic fatty acid sources to total VLDL-TG production did not differ between sexes ($P = 0.539$): systemic plasma FFA contributed 78 ± 15% of fatty acids in VLDL-TG in men and 75 ± 7% in women; nonsystemic fatty acids accounted for 22 ± 15 and 25 ± 7% of fatty acids in VLDL-TG in men and women, respectively. The absolute secretion rates of VLDL-TG derived from systemic plasma FFAs and nonsystemic fatty acids were therefore both significantly greater in women than in men ($P = 0.040$ and $P = 0.035$, respectively; Fig. 2).

The plasma clearance rate of VLDL-TG was much greater in women than men (by ~70%, $P = 0.015$), whereas the plasma clearance rate of VLDL-apoB-100 was not different between sexes ($P = 0.943$; Fig. 3). The MRT of VLDL-TG was approximately 45% shorter ($P < 0.001$) and the MRT of VLDL-apoB-100 was approximately 25% shorter ($P = 0.041$) in women than men (Fig. 3).

VLDL-TG and VLDL-apoB-100 secretion rates did not correlate with plasma FFA concentration, but the molar ratio of VLDL-TG and VLDL-apoB-100 secretion rates was negatively associated with VLDL-TG MRT ($R^2 = 0.684$; $P < 0.001$, Fig. 4).

Discussion

The regulation of hepatic VLDL secretion into plasma is of considerable clinical importance because derangements in VLDL metabolism are linked to CVD (2–4). The data from the present study indicate that there are considerable sex differences in VLDL metabolism. Increased VLDL-TG clearance from the circulation, which offsets increased hepatic VLDL-TG secretion in women, compared with men, is responsible for lower plasma VLDL-TG concentration in women, whereas decreased hepatic VLDL-apoB-100 secretion, combined with shorter VLDL-apoB-100 residence time in the circulation, is responsible for lower plasma VLDL-

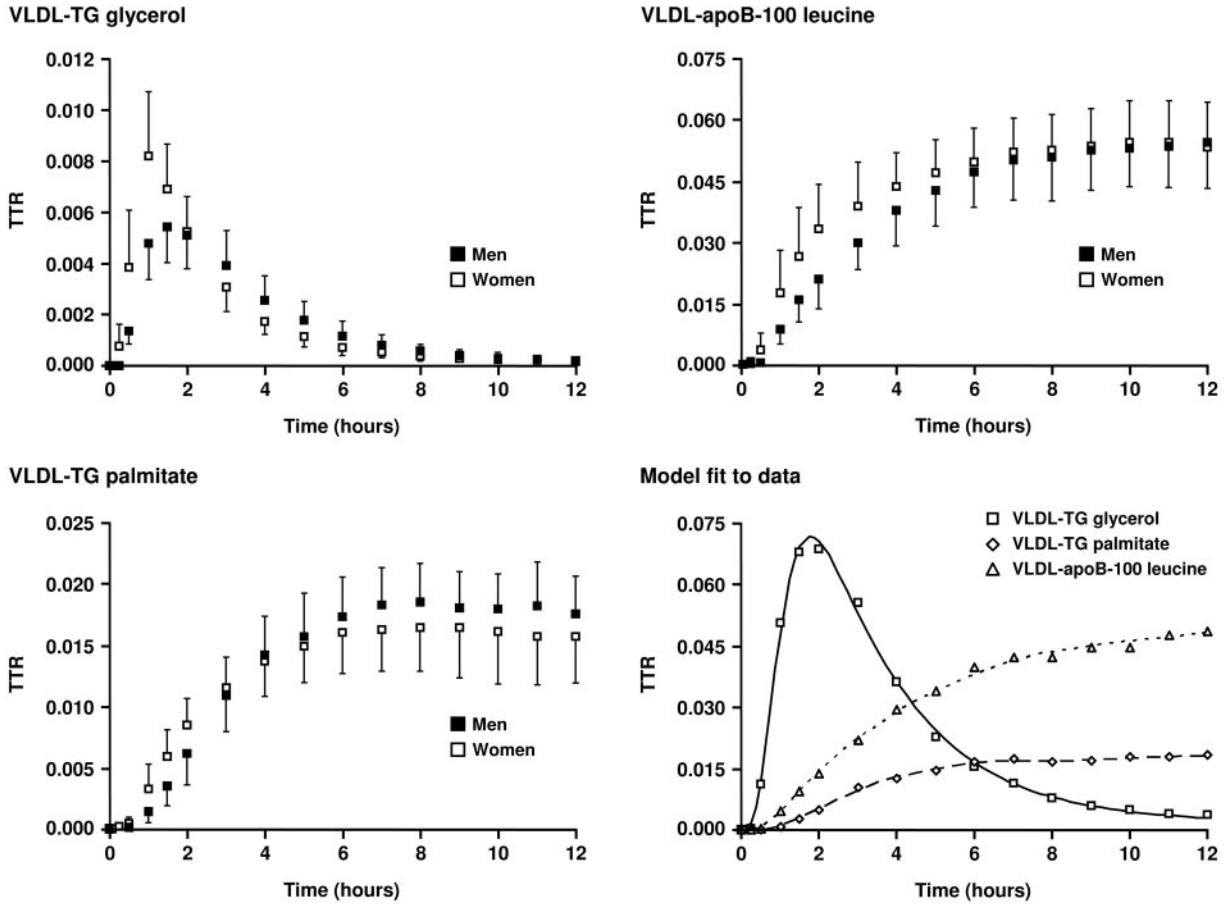

FIG. 1. Average (means ± SD) TTR time courses for glycerol in VLDL-TG (*top left panel*), palmitate in VLDL-TG (*bottom left panel*), and leucine in VLDL-apoB-100 (*top right panel*) in men and women and a representative model fit to the TTR data from one subject for VLDL-TG glycerol (scaled ×15 for presentation), VLDL-TG palmitate, and VLDL-apoB-100 leucine (*bottom right panel*).

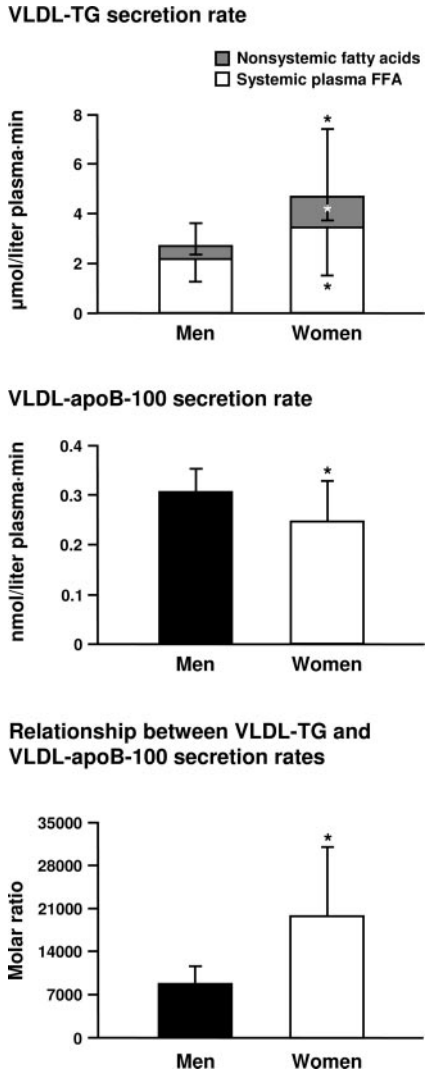

FIG. 2. VLDL-TG (*top panel*) and VLDL-apoB-100 (*middle panel*) secretion rates into plasma and molar ratio of VLDL-TG and VLDL-apoB-100 secretion rates (*bottom panel*), an index of the TG content of VLDL that are secreted by the liver, in men and women. Data are presented as means ± SD. For VLDL-TG secretion, the contribution of systemic plasma FFA to fatty acids in VLDL-TG is represented by the *white bars*, and the contribution of nonsystemic fatty acids to fatty acids in VLDL-TG is represented by the *gray bars*. *Error bars* pointing upward represent SDs for total VLDL-TG secretion rate, and *error bars* pointing downward represent SDs for VLDL-TG derived from systemic plasma FFAs and nonsystemic fatty acids. *, Value in women is significantly different from corresponding value in men; $P < 0.05$.

apoB-100 concentration in women than men. Moreover, a greater molar ratio of hepatic VLDL-TG and VLDL-apoB-100 secretion rates in lean women than lean men suggests that the liver in women secretes VLDL particles that contain more TGs, and are therefore larger, than VLDL secreted by the liver in men. This provides a potential mechanism responsible for increased VLDL-TG plasma clearance and consequently shorter VLDL-TG MRT and likely also VLDL-apoB-100 MRT in women because results from studies *in vitro* and *in vivo* in human subjects demonstrate faster removal of TGs from large, TG-rich VLDL particles than small, TG-poor particles (20, 21, 28).

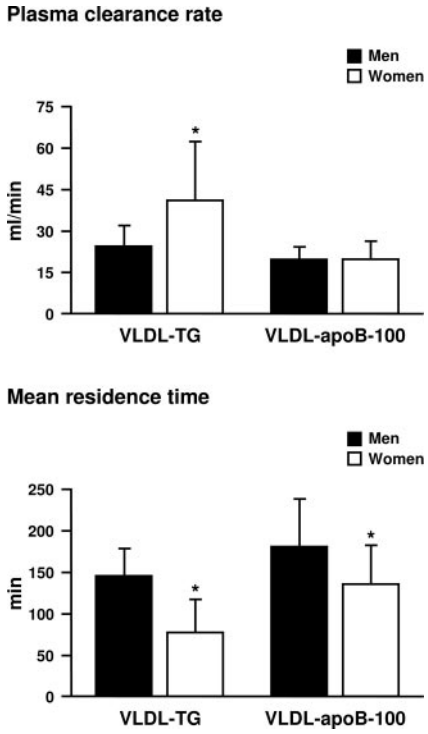

FIG. 3. VLDL-TG and VLDL-apoB-100 plasma clearance rates (*top panel*) and mean residence times (*bottom panel*) in men and women. Data are presented as means ± SD. *, Value in women is significantly different from corresponding value in men; $P < 0.05$.

Our findings indicate that the relationship between VLDL-TG and VLDL-apoB-100 secretion differs between healthy, lean men and women during basal conditions. A dissociation in hepatic VLDL-TG and VLDL-apoB-100 secretion rates has previously been demonstrated in response to physiological perturbations, such as dietary interventions (15, 17, 18) and endurance exercise (16). Therefore, different physiological mechanisms must be responsible for regulating VLDL-TG and VLDL-apoB-100 secretion. This metabolic flexibility provides a mechanism that allows the liver to safely handle an increased influx of fatty acids from the bloodstream or intrahepatic fatty acid sources by altering the TG content of secreted VLDL. Our study is the first demonstration that the molar ratio of VLDL-TG and VLDL-apoB-

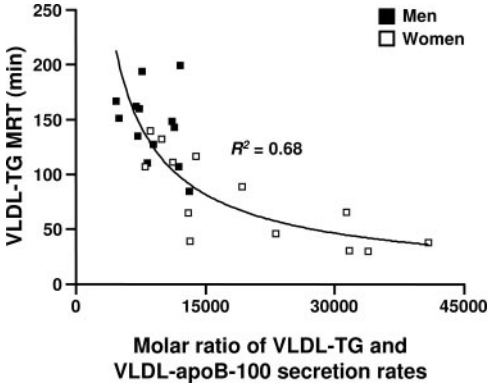

FIG. 4. Relationship between the molar ratio of VLDL-TG and VLDL-apoB-100 secretion rates and the MRT of VLDL-TG in the circulation.

100 secretion rates is greater in women than men. Although we did not assess the kinetics of VLDL subfractions (*e.g.* large, TG rich $VLDL_1$ and small, TG poor $VLDL_2$), most of the additional TG secretion in women was probably due to increased TG content, rather than secretion of an increased number, of large, TG-rich $VLDL_1$ particles because the bulk (~90%) of VLDL-TG is secreted in large, TG-rich $VLDL_1$ (29, 30), and women in our study secreted overall fewer VLDL particles (large and small combined) than men. However, we cannot exclude secretion of fewer small, TG-poor $VLDL_2$ in women, compared with men. These findings are in agreement with the observed sex differences in the size of VLDL particles secreted by the liver from rodents *in vivo* (31) and *ex vivo* (32, 33).

Our kinetic data suggest that women secrete larger, TG-richer VLDL into the circulation than men. However, data from studies that measured plasma VLDL particle size indicate that on average VLDLs in women are smaller than VLDLs in men (6, 8). Nonetheless, these results are not necessarily inconsistent with each other. The size of circulating VLDL reflects the balance between the number and TG content of VLDL secreted into the circulation and the rate of TG removal from circulating VLDLs via LPL-mediated lipolysis, neutral lipid exchange with other lipoproteins (*e.g.* HDLs), conversion of VLDL to smaller, denser lipoprotein particles (*e.g.* IDLs and LDLs), and direct uptake of VLDLs by tissues. Based on our data, the smaller size of circulating VLDL in women than men (6, 8) is probably, at least in part, due to more efficient VLDL-TG removal from the circulation [(34, 35) and present study] because of the greater molar ratio of hepatic VLDL-TG and VLDL-apoB-100 secretion rates. *In vitro* studies have demonstrated that LPL-mediated lipolysis of TGs in large, TG-rich lipoproteins is more efficient than lipolysis of TGs in small, TG-poor particles (20, 21). The strong negative relationship between the molar ratio of VLDL-TG and VLDL-apoB-100 secretion rates and VLDL-TG MRT in our study is in good agreement with this hypothesis.

Sex differences in LPL activity may have further augmented the difference in VLDL-TG plasma clearance rate between our men and women because heparin-releasable LPL activity (an index of lipolytically active, endothelial-bound LPL) in adipose tissue (36–38) and skeletal muscle (39) was found to be greater in lean women than lean men. Some of the sex difference in VLDL-TG removal from plasma was probably also due to differences in the conversion of VLDL to IDLs and LDLs or direct uptake of VLDLs by tissues because VLDL-apoB-100 MRT was shorter in women than men. The fact that the difference in VLDL-TG MRT between men and women was much greater (~45%) than the difference in VLDL-apoB-100 MRT (~25%), however, indicates that this route of TG removal accounted for only a portion of the difference in total VLDL-TG removal. This is probably related to the original TG content of VLDLs at the time of secretion, which was more than double in women than men; hence, the particles remained in the VLDL density range despite faster loss of core TG. Furthermore, we cannot exclude differences in TG removal between men and women due to differences in the rate of neutral lipid exchange. However, it is conceivable that the shorter residence time of VLDL particles in the circulation in women than men may have actually limited the exchange of TGs from VLDLs and cholesterol esters from HDL and thereby provide a potential mechanism responsible for higher plasma HDL cholesteryl concentration in women than men (5, 6, 8).

The mechanisms responsible for the differences in VLDL-TG and VLDL-apoB-100 kinetics between men and women are not clear. Hepatic FFA availability from plasma and circulating insulin are considered to be the major physiological regulators of hepatic VLDL-TG and VLDL-apoB-100 secretion (13, 40). Increasing plasma FFA concentration by infusing a lipid emulsion plus heparin stimulates, whereas insulin infusion suppresses both VLDL-TG and VLDL-apoB-100 production (14). In our subjects, FFA Ra adjusted for differences in body size, FFM, and FFA volume of distribution was significantly greater, and plasma FFA concentration tended to be greater in women than men. This could have stimulated VLDL-TG secretion but cannot explain the lower rate of VLDL-apoB-100 secretion in women. The fact that we did not measure fatty acid oxidation does not alter the interpretation of our results because the basal rate of fat oxidation, even when adjusted for body mass and/or FFM, is lower or not different in women, compared with men (41). In addition, women had less intraabdominal fat than men, whereas the secretion of VLDL-TG from nonsystemic fatty acids, predominantly derived from intraabdominal fat depots, was much greater in women than men. Although our findings appear to contradict the commonly accepted notion that FFA availability is an important regulator of VLDL-TG and VLDL-apoB-100 secretion (13, 14, 42, 43), they are in agreement with several recent studies that reported no association between plasma FFA availability and VLDL-TG (16, 29, 44–47) or VLDL-apoB-100 (16, 48, 49) secretion rates. It is also unlikely that insulin contributed to the sex differences in VLDL-TG and VLDL-apoB-100 kinetics because plasma insulin concentration and whole-body insulin sensitivity, determined with the HOMA-IR index, were not different between our men and women. Although we cannot exclude sex differences in hepatic insulin sensitivity, it is unlikely that such differences would have affected the conclusions from our study because insulin suppresses both VLDL-TG and VLDL-apoB-100 secretion rates *in vivo* (14), but VLDL-TG secretion was greater, whereas VLDL-apoB-100 secretion was lower in our women than our men.

The absence of obvious differences in known systemic regulators of VLDL-TG and VLDL-apoB-100 secretion suggests that sex differences in hepatic VLDL-TG and VLDL-apoB-100 secretion may be directly related to differences in sex hormones between men and women. However, administration of estrogens or estrogens and progestogens stimulates both VLDL-TG (50, 51) and VLDL-apoB-100 (52–55) secretion rates, whereas only VLDL-TG but not VLDL-apoB-100 secretion rate was greater in our women than our men. Therefore, other gonadal hormones or cellular and metabolic adaptations to lifelong exposure to the sex-specific hormonal milieu are responsible for at least some of the differences in VLDL-TG and VLDL-apoB-100 metabolism between men and women. This notion is supported by data from studies involving isolated liver tissue: perfused livers from female rats produce more VLDL-TG than those from male rats (32, 33, 56, 57), and gene expression of hepatic enzymes involved

in intracellular lipid synthesis are up-regulated, whereas those involved in fatty acid oxidation are down-regulated in livers from female, compared with male rodents (58–60).

In summary, the data from this study elucidate the metabolic mechanisms responsible for the differences in VLDL-TG and VLDL-apoB-100 concentrations between healthy, lean men and women. Increased VLDL-TG clearance from the circulation, which offsets increased hepatic VLDL-TG secretion in women, compared with men, is responsible for lower plasma VLDL-TG concentration in women, whereas decreased hepatic VLDL-apoB-100 secretion, combined with shorter VLDL-apoB-100 residence time in the circulation, is responsible for lower plasma VLDL-apoB-100 concentration in women than men. In addition, differences in the relationship between hepatic VLDL-TG and VLDL-apoB-100 secretion rates indicate that the liver in women secretes TG-richer VLDL than the liver in men, which may in part be responsible for the greater VLDL-TG plasma clearance and shorter VLDL residence time in the circulation in women. Sex differences in hepatic secretion of VLDL-TG and VLDL-apoB-100 are not readily explained by FFA availability, insulin, or the direct action of the predominant sex hormones, suggesting there is sexual dimorphism in regulatory factors intrinsic to the liver.

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